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Double Mutant Cycle Analysis: High through Output.

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Experimental DMC studies have usually focused on specific pair wise interactions in systems of interest. Given their relatively small scale nature and inherent bias in choice of mutations, they have been unable to shed light on more global features of proteins such as all osteric networks and statistical tendencies for positive and negative epistasis, that is the prevalence and nature of coupling of residue pairs in different proteins. Consequently, there has been a need for studies in which the effects of very large numbers of mutations on some thermodynamic or kinetic property of a protein are measured quantitatively. In an early such study, 2048variants of l-repressor were generated in which the residues comprising the helix-turn-helix motif of its N-terminal domain were mutated to alanine with a single site probability of 0.5. Comparing the frequencies of pair wise substitutions with the product of the corresponding single- site substitution frequencies in active variants revealed that most of the mutational effects are additive (the Boltzman n distribution relates frequenciest of reeenergies). Recently, all possible DMCs were constructed for the9 residue a2 helix off different PDZ domains, there by generating 13168variants (1wild-type, 19single mutants and 36×19×19 double mutants per domain. A bacterial two-hybrid assay was the used to determine the binding free energies of the PDZ variants for their respective peptide ligands. The values of the coupling energies for most pairs of positions in all the homologs were found to have unimodal distributions centered close to zero, there by indicating wide spread additive effects as reported before. It remains to be established, however, whether the average of the coupling energies for all possible 1919combinations of mutations at a pair of positions used in this study provides a better estimate of the pair wise coupling energy in the wild-type protein than a value derived from the effects of judiciously chosen mutations (eg. toalanine). The bimodal distributions observed were interpreted to reflect the existence of equilibrium between two distinct conformational states of the PDZ domains. Interestingly, the different PDZ domains display some variation in their couplings but they also have a set of conserved couplings that may reflect common functional constraints. It should be noted that coupling energies for intra-protein in

Teractions determined by measuring binding free energies have a different physical origin than those determined by measuring folding free energies. Massive DMC analysis of protein–protein interactions has also been carried out for protein G domain B1(GB1)binding to the F Cdomaino fIgG(IgGFC) and the formation of the AP-1transcription factor complex by the products of the FOS and JUN proto-oncogenes. In the latter case, all possible substitutions were introduce dat32 positions in each of the proteins, there by creating369664(19 19 32 32)inter-protein DMCs. Data were obtained for 107625 of the cycles using a protein complementation assay in which the two proteins of interest, JUN and FOS, were fused to two respective fragments of methotrexate-resistant DHFR. Binding of the proteins to each other brings the two fragments together and allows growth, in the presence of methotrexate, that reflects the strength of the protein–protein interaction(PPI). Large-scale additivity was observed also in this study, that is the effects of double mutations on the PPI scores were well predicted by the product of effects of the corresponding single mutations. Given, however, that the PPI score is not a well-defined thermodynamic prop-erty, additivity was also demonstrated by using a model that relates the PPI score to binding free energy in a rigorous manner. In agreement with the study on the GB1–IgGFC interaction, negative epistasis was found to be more frequent than positive epistasis and was observed for 15 and 11% of the pairs tested, respectively. Negative epistasis was usually found to be observed when the two single mutations reduced the PPI strength in a moderate fashion where as positive epistasis when the two single mutations greatly weakened the interaction or had opposing effects. A more dramatic difference between the frequencies of negative and positive epistasis was found in a study on a 9-residue substrate-binding motif of yeast hsp90. There, about 46 and 2% of the studied pairs were found to display.

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